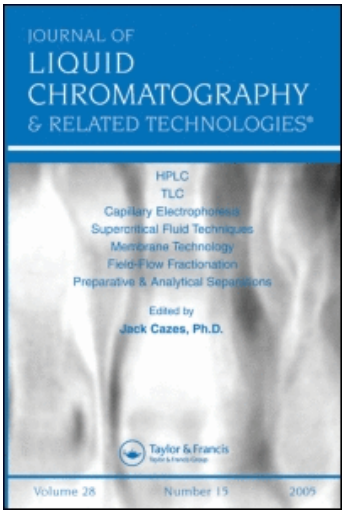

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A Novel Approach to Prepare a Glass-Fiber-Packed Capillary Column for Capillary Electrochromatography

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Abstract: In the present study, a novel method was proposed to pack long glass fibers into a narrow-bore capillary for capillary electrochromatography (CEC) applications. The factors that influence the packing process were studied in detail. The results demonstrated that solvents played dual roles to introduce the glass fiber into the capillary during the packing process, a lubricant and a carrying reagent. A preliminary CEC performance, between a glass fiber-packed-C_{18}-bonded open tubular column and a C_{18}-bonded open tubular one, was compared. The former exhibited a 25–60% larger electroosmotic flow (EOF) than that of the latter. High separation efficiency of toluene and naphthalene was obtained on the fiber-packed column, while the separation time decreased about 19%. The glass fiber-packed column showed a promising perspective in CEC applications, due to its high EOF, relative low phase ratio, ease of preparation, and tunable selectivity.

Keywords: CEC, Fast analysis, Glass fiber, Packing technology

INTRODUCTION

Capillary electrochromatography (CEC), which combines the desirable features of high selectivity of liquid chromatography (LC) and high efficiency of capillary electrophoresis (CE), has become considerably popular.[1] Generally, the columns used in CEC can be classified into

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three categories: (1) open tubular columns,[2,3] (2) packed columns,[4–6] and (3) monolithic columns.[7–15]

Open tubular capillaries can be easily prepared and operated without bubble formation. Nevertheless, low sample capacity and low detection sensitivity originating from the short optical path length prevent it from being widely used.[16] Packed columns may be a solution to these problems. However, it still meets difficulties during the packing and frit making process.[17] Therefore, much effort is devoted to the development of monolithic columns. The superiority of monolithic columns is obvious: it can be formed in situ; the properties such as porosity, surface area can be tailored, and retaining frits are not required. One disadvantage of it may be the difficulty of manufacturing,[18] especially for silica-based monoliths. However, it is still important to explore new stationary phases in CEC for its development.

Recently, Jinno et al. have attempted a novel packing material, long fibrous cellulose acetate (CA) in CEC.[19,20] Compared with traditional CEC columns, this new kind of column has its merits and shows potential use in CEC. The packing procedures of the new columns are simpler than those particle packed ones or monolithic ones. The reproducibility between columns can, therefore, be guaranteed. Although a relative wide-bore capillary was used, it can provide the similar phase ratio as that of a narrow-bore open tubular capillary. However, the detection path length of the former is longer than that of the latter, which affords higher sensitive detection. Unfortunately, due to the characteristic limits of CA fibers as well as large internal diameter (i.d. $\geq 200\,\mu m$) of the capillary utilized, the column efficiency is limited.

Herein, we adopted glass fibers as packing material and put forward a new technique for the packing process. The new packing technique facilitates the use of narrow-bore capillaries, which dramatically increased the column efficiency. Fast separation and suitable phase ratios could be obtained.

EXPERIMENTAL

Chemicals, Materials, and Instrumentation

The fused-silica capillary was obtained from Hebei Yongnian Optical Fiber Plant (Hebei, China). Glass fibers with the length of 38 cm and diameter of 15 μm were purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). Octadecyltriethoxysilane (C_{18}-TEOS) was from the Chemical Factory of Wuhan University (Wuhan, China). Other chemicals of analytical grade were from Shanghai General Chemical Reagent Factory (Shanghai, China).

A vacuum pump (water recycling pump) was from Yuhua Co. Ltd. (Henan, China). The vacuum degree is ca. 10^{-3} torr. CEC experiments were

performed at room temperature on a CAPEL 105 Capillary Electrophoresis System (LUMEX, St. Petersburg, Russia) equipped with a UV-Vis detector.

Preparation of C_{18}-Bonded Open Tubular Capillary (C_{18}-Column)

Fused-silica capillaries of 50 μm i.d. were activated first. Thereafter, it was filled with 10% (v/v) solution of C_{18}-TEOS in anhydrous toluene to react at 383 K for 10 h. The column was then washed with toluene, acetone, and methanol, in that order. A detection window was made by eroding the polymer coating.

Glass Fiber Packing Process

Glass fiber was washed with concentrated hydrochloric acid, distilled water, and acetone to remove any possible residue on the fiber's surface.

The C_{18}-Column was flushed with a suitable solvent for half an hour first. And then one tip of the pretreated glass fiber was inserted into the hollow capillary (inlet) under a microscope. Thereafter, the capillary tip holding the glass fiber (inlet) was immersed in the solvent, while the other end of the capillary (outlet) was connected to a vacuum pump, as the setup described in Figure 1. Under vacuum, the glass fiber went into the hollow capillary gradually. When the fiber came out of the outlet of the capillary, the vacuum pump was stopped immediately. A permeable urea-formaldehyde resin was utilized to immobilize the glass fiber in-situ at the outlet of the capillary. The column prepared was named fiber-packed-C_{18}-bonded capillary column (FP-C_{18}-Column).

CEC

Buffer solutions were prepared and filtered through a G-4 fritted-glass funnel before use. The samples were introduced into the column by electrokinetic injection at 2 kV for 2 s. On column detection wavelength was 254 nm. Thiourea was used as a neutral marker. The CEC apparatus and capillaries were conditioned with the running buffer for 2 h before daily operation.

RESULTS AND DISCUSSION

Choice of Fiber

Although the availability of fibers is abundant, those for the purpose of CEC packing seem to be limited because of various defections such as

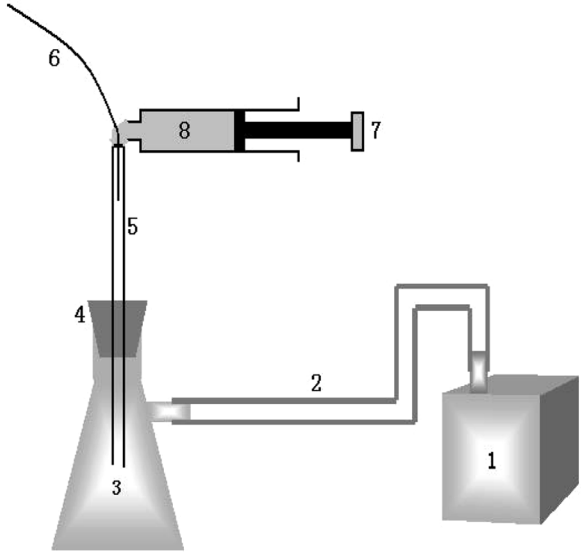

Figure 1. The schematic diagram of the glass fiber packing apparatus: 1- vacuum pump, 2- connection tube, 3- filtering flask, 4- sealing plug, 5- capillary, 6- glass fiber, 7- solvent container, 8- solvent.

low strength, short dimension, and difficulty of modification. Among them, glass fibers seem an ideal choice. First, plenty of silanols on the fiber's surface can supply high electroosmotic flow (EOF) in CEC. Secondly, the high reacting activity of silanols can facilitate further surface modification to afford high selectivity, in addition to the surface modification on the capillary. Thirdly, the pore structure and the surface area of glass fibers can be easily tailored by base treatment or hydrofluoric acid eroding. By packing glass fibers into a capillary, the phase ratio (Vm/Vs) of the column can be sharply decreased, comparing to that of unpacked one (demonstrated below).

The Fiber Packing Process

Until now, the commonly used method for fiber packing is assisted by a high strength thread, which can easily load into the hollow capillary. The thread was first tied to the tip of a fiber. Under the thread guiding, the fiber was pulled out through the capillary.[19,20] Owing to the operational inconvenience and the restriction of the dimension of the thread, until now, the smallest bore of capillaries used for this purpose is 200 μm i.d. It is difficult to introduce the fiber into a narrow-bore

capillary, which is normal for CE and CEC. It has been certified that wide-bore capillary is not suitable for CEC separations due to the high electric current as well as the low column efficiency.[21–26] To enhance the column efficiency and further develop the CEC in this mode of fiber-packing column, a narrow-bore capillary of 50 or 75 μm i.d. would be more attractive.

The proposed method, herein, for packing fibers into narrow-bore capillaries is named the vacuum dragging-solvent carrying method. Figure 1 shows the schematic diagram of the fiber packing setup. It is composed of a vacuum pump, a filtering flask, and a solvent container. The vacuum pump acted as the dragging force provider while the solvent served as a lubricant, as well as a carrying reagent.

The solvent is vital to achieve the successful packing. Without solvent, the fiber could only move several centimeters into the capillary under vacuum dragging, and then it would be stopped. But if a solvent was adopted, the fiber's packing was smooth and fast. Apparently, the solvent made an effect in the packing process.

By wetting both the fiber and the capillary's surface, the solvent decreased the frictional force between them, acting as a lubricating reagent. On the other hand, as a rheological fluid, the vacuum dragging force made the solvent rush into the capillary much faster than the fiber (the solid) did, which may produce a pro frictional force (between the solvent and the fiber) to assist the fiber's moving into the capillary. Obviously, the solvent also played a carrying role there.

Further studies revealed that the nature of solvent was also very crucial. Three kinds of solvents including acetone, methanol, and water were attempted in our experiments. It could be observed that packing was the fastest in the case of acetone used, while the slowest in the case of water. Probably, the viscosity of the solvents was responsible for this observation. Under constant vacuum pump force, the solvent with high viscosity migrates at a relative lower velocity than those with low viscosity. The viscosities of acetone, methanol, and water are 0.30 cP, 0.54 cP, and 0.89 cP (398 K), respectively. Therefore, of the three, the migration velocity of acetone is the highest. The fast movement of it would produce persistent frictional force to the fiber, which facilitates the fiber's loading into the capillary, so the fiber can be packed very quickly. Although water can provide much a better lubricating effect than acetone because of its higher viscosity, the fiber's loading is not as fast. Obviously, the main role of the solvent is a carrying reagent.

In comparison with the traditional thread guiding method, the vacuum dragging-solvent carrying method is much simpler and more applicable. Without thread guiding, the new method can be used in packing fibers into capillaries with small bores, which may contribute much to the development of this kind of new CEC column. In fact, using acetone

as the carrying solvent, 10 min. is enough for totally loading a fiber (15 μm i.d.) of 38 cm length into the capillary of 50 μm i.d.

EOF Comparison

The electroosmotic mobility, μ_{eof}, and EOF linear velocity of the eluent, ν_{eo}, can be expressed by the following equation:

$$\mu_{eof} = \delta\sigma/\eta \tag{1}$$

$$\nu_{eo} = \mu_{eof} * E \tag{2}$$

Where δ, σ, η, E are the electrical double layer thickness, the surface charge density, the viscosity of the eluent and the applied electric field strength, respectively.[27]

In comparison to open tubular columns, one of the great advantages of the glass fiber packed column may lie in that it can sharply increase the EOF. Figure 2 shows curves of EOF linear velocity (ν_{eo}) vs the external electric field strength (E) on C_{18}-Column and FP-C_{18}-Column. Obviously, with increasing E, the ν_{eo}s of both columns showed a linear increasing trend. Figure 3 is the pH effect on μ_{eof}. As the pH in the mobile phase increased, the ionization of silanol groups on the surface of the fiber and inner wall of the capillary increased simultaneously, resulting in the increase of σ in Equation 1 and thus increasing the μ_{eof} accordingly. Figure 4 shows the effect of the methanol content of the mobile phase

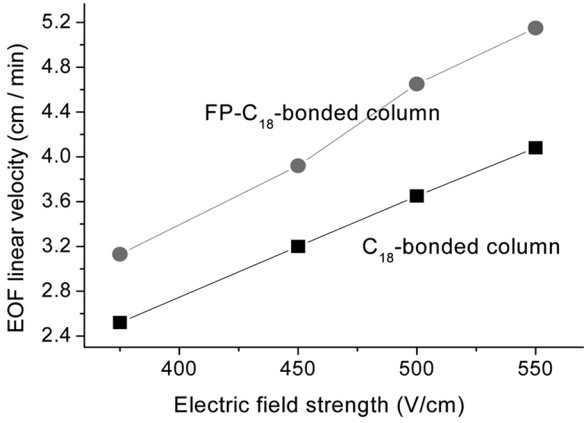

Figure 2. Comparison of EOF linear velocities under different electric field strength for (●) FP-C_{18}-Column and (■) C_{18}-Column. Conditions: 38 cm × 50 μm i.d. (effective length 21 cm); pH 7.0; electrolyte, 5mmol · L^{-1} phosphate + methanol (70/30, v/v).

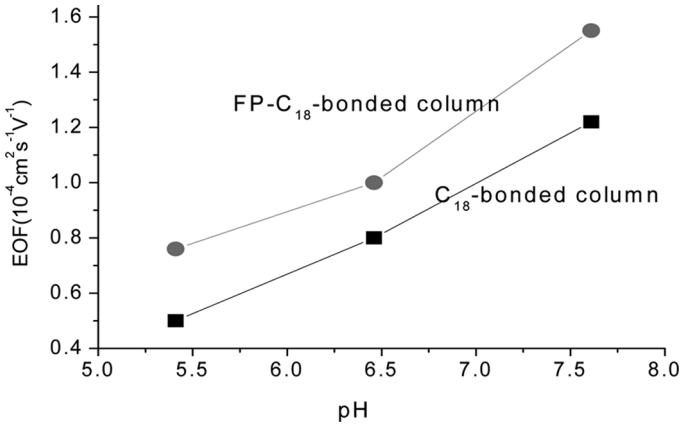

Figure 3. Comparison of the influence of pH values on EOFs for (•) FP-C_{18}-Column and (■) C_{18}-Column. External field strength, 500 V/cm. Other conditions are the same as in Figure 2.

on the μ_{eof} of the two columns. With increasing methanol content, both of the μ_{eof} decreased. It may be due to the following reasons: the increase in methanol content decreases the ionization of silanol groups on the surface of the fiber and inner wall of the capillary, and hence a decrease in σ in Equation 1, resulting in the decrease in μ_{eof}. Additionally, the viscosity of the eluent increases with the increasing methanol content from 15% to 30% (v/v). Consequently, the μ_{eof} decreased because of the inverse proportion of μ_{eof} to the eluent viscosity (as shown in Equation 1).

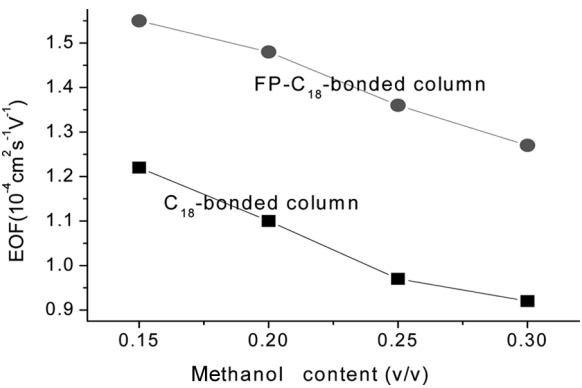

Figure 4. Comparison of the influence of methanol content on the EOFs for (•) FP-C_{18}-Column and (■) C_{18}-column. Electrolyte, 5 mmol $\cdot$ L^{-1} phosphate, pH 7.0. Other conditions are the same as in Figure 3.

It can be obviously found from Figure 2 to Figure 4 that, the FP-C_{18}-Column shows 25%–60% greater μ_{eof} than that of C_{18}-Column under the same condition. The increase is probably ascribed to the difference of charge density in the two columns. In the C_{18}-Column, a great part of the surface silanols was covered with C_{18} ligand. The remaining free silanols were very limited, which resulted in low charge density after ionization, and thus low EOF. After fiber packing, because of the abundant silanol groups on the fiber's surface, the charge density can be sharply increased. According to Equation 1, the μ_{eof} will be correspondingly increased. Therefore, the μ_{eof} of the FP-C_{18}-Column is faster than that of the C_{18}-Column. This characteristic may render the fiber-packed column as a solution to fast analysis and separation in CEC applications.

CEC Performance

Toluene and naphthalene were utilized for the preliminary evaluation of the FP-C_{18}-Column performance. For comparison, their separation on the C_{18}-Column was also performed. Figure 5 (a) and (b) were the electrochromatograms of their separation on these two columns,

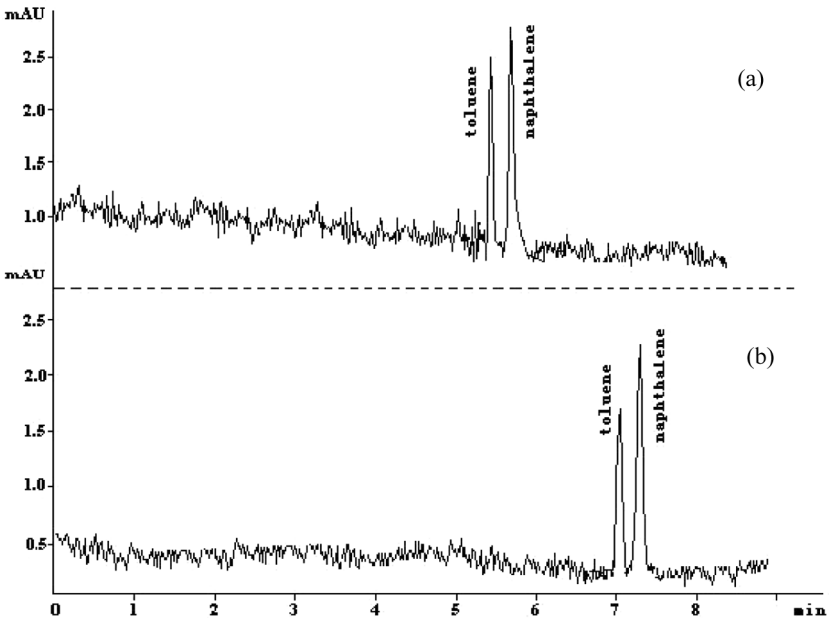

Figure 5. Separation of toluene and naphthalene on (a) FP-C_{18}-Column and (b) C_{18}-Column. Conditions: capillary, 38 cm × 50 μm i.d. (effective length 21 cm); mobile phase, 5 mmol · L^{-1}phosphate + methanol (80/20, v/v), pH 8.0.

respectively. Apparently, the tested compounds can be baseline separated on both columns, and their column efficiencies were in the same magnitude, around 150,000–170,000 plates per meter for toluene. However, Figure 5 manifested that the separation speed was sharply increased after fiber loading. The separation time reduced about 19%, from 7.2 min on the C_{18}-Column to 5.8 min on the FP-C_{18}-Column. Obviously, the increasing μ_{eof} on the FP-C_{18}-Column contributed to this difference.

Stability and Reproducibility

μ_{eof} was measured to evaluate the stability of the fiber-packed column (conditions: thiourea was the EOF marker and the buffer was 5 mM phosphate of pH 7.3). The relative standard deviations (RSDs) of day-in-day and day-to-day were 1.2% (n = 10) and 2.3% (n = 16), respectively. The stability of the fiber-packed column was excellent.

The reproducibility experiment was performed in the same conditions, but on different batches of fiber-packed columns. The column-to-column RSD was around 3.2% (n = 5). Considering the small dimension size of the columns as well as the difference of the fibers and capillaries, the variation is reasonable and acceptable.

CONCLUSIONS

A novel method, named vacuum dragging-solvent carrying, was developed for packing glass fibers into a small bore capillary column in the present work. Experiments revealed that solvents play an important role in the packing. Compared to the traditional capillary electrochromatography (CEC) columns, the new column is fritless and easy to handle, whose selectivity and electroosmotic flow (EOF) can be easily tailored through modification either or both of the capillary's and the fiber's surface.

In order to determine the effectiveness of this method, a narrow-bore capillary was chemically bonded with C_{18} for affording selectivity while a fiber was activated to provide the EOF. The studies demonstrated that the new column is characterized by high μ_{eof} as well as high resolution. High efficiency and fast separation of toluene and naphthalene was accomplished, which obviously discloses the perspective of this kind of new CEC column. It is highly anticipated to be a complementary to the present available CEC columns. More studies about tuning the selectivities of the glass fiber and/or capillary are undergoing.

ACKNOWLEDGMENTS

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